

*Sub  
A'*

**Claims:**

1. A tissue culture method for cultivation of marine algae, said method comprising the steps of:

5        a) establishing axenic viable material of an algae for tissue culture by sequential treatment of the algal material in sterile sea water supplemented with domestic liquid detergent, povidine iodine and finally incubating the treated material in Provasoli enriched seawater (PES) medium with a broad spectrum antibiotic mixture and a fungicide for about 24 to 96 hours followed by thorough cleaning with sterile sea water to remove any traces of antibiotics and fungicide and blotting with sterile filter paper to obtain axenic explants;

10        b) culturing the axenic explants on agar plates fortified with PES medium at a temperature ranging between 20-25°C in the presence of cool white fluorescent lights at about 20-50  $\mu$  mol photons  $m^{-2} s^{-1}$  irradiance and a 12 : 12 light and dark cycle for induction of callus;

15        c) excising the callus from the explant after a period of at least 40 days and subculturing the calli on fresh agar plates in the presence of cool white fluorescent lights with 40-60  $\mu$  mol photon  $m^{-2} s^{-1}$  irradiance and a 12 : 12 light and dark cycle to obtain differentiated densely pigmented oval or spherical shaped micro-propagules;

20        d) subculturing thin slices of the pigmented callus in agar plates in Provasoli Enriched Seawater (PES) medium containing plant growth regulators, for a period of about 20 to 40 days, in the presence of cool white fluorescent lights of 40- 60  $\mu$ mol photon  $m^{-2} s^{-1}$  irradiance and a 12 : 12 light and dark cycle to achieve enhanced somatic embryogenesis and micro-propagule formation in pigmented filamentous callus;

25        e) transferring the filamentous calli with somatic embryos to liquid PES medium in agitated condition for morphogenesis and development of young plantlets with multiple shoots from propagules; and

25 f) cultivating algal biomass on a large scale by growing the young plantlets in enclosed perforated polythene bags.

5 2. A method as claimed in claim 1 wherein the material for tissue culture is selected from the group of Rhodophytic and Phaeophytic marine algae comprising the genera *Eucheuma*, *Gigartina*, *Chondrus*, *Laminaria*, *Undaria*, *Ecklonia*, *Eisenia*, *Macrocystis*, *Sargassum* and *Turbinaria*.

10 3. A method as claimed in claim 1 wherein the material for tissue culture is selected from the group of algae comprising *Eucheuma striatum*, *Kappaphycus alvarezii*, *Eucheuma cottonii*, *Eucheuma denticulatum*, *Eucheuma spinosum*, *Eucheuma alvarezii*, *Eucheuma procrusteanum*, *Gigartina intermedia*, *Gigartina exasperata* and *Chondrus crispus*.

15 4. A method as claimed in claim 1 wherein the axenic explants comprise 1 to 6 mm long cuttings with 3-4 mm diameter and selected from the upper distal parts of the algae.

20 5. A method as claimed in claim 1 wherein the algal material is treated first with 0.1-1% domestic liquid detergent for 5 to 20 minutes, followed by treatment with 0.1-2% providine iodine for 2 to 7 minutes, and finally in provasoli enriched seawater with 1-5% antibiotic mixture for 04-96 hrs.

25 6. A method as claimed in claim 1 wherein the antibiotic mixture comprises penicillin, streptomycin sulphate, kanamycin, nystatin and neomycin in 100 ml distilled water.

7. A method as claimed in claim 1 wherein the axenic explants are cultured on agar plates containing 0.8-3% agar medium fortified with provasoli enriched seawater at 20-25°C in the presence of cool white fluorescent light at 20-50  $\mu$  mol photon  $m^2 s^{-1}$  with a 12:12 light and dark cycle at 20-25 °C.

*Sub*

*A<sup>2</sup>*

8. A method as claimed in claim 1 wherein the calli is subcultured by growing thin slices of pigmented calli as embedded cultures in agar plates containing 0.3-0.6% agar and made in provasoli enriched seawater medium at 20-25°C in the presence of cool white fluorescent light at 20-50  $\mu$  mol photon  $m^2 s^{-1}$ , irradiance with 12:12 dark and light cycle to obtain profusely branched filamentous pigmented calli in each embedded block.

9. A method as claimed in claim 1 wherein the plant growth regulators are selected from 0.1-1.0 mg/l naphthalenacetic acid and 0.1mg  $l^{-1}$  each of naphthalenacetic acid and 6-benzylaminopurine.

10

10. A method as claimed in claim 1 wherein the axenic explants in step (b) are cultured on agar plates for a period of about 40-45 days.

11. A method as claimed in claim 1 wherein the algal biomass in step (f) are grown in 60 x 45 cm polythene bags attached to long floating lines in the sea and the crop is harvested after a period of about 60 days.

12. A method as claimed in claim 1 wherein the young plantlets at step (f) are cultured in perforated polythene bags with annual seawater temperature ranging from 22.5°C - 28.5°C, pH from 7.81 - 8.26, salinity from 24.0 ‰ - 34.0 ‰, dissolved oxygen from 7.84 ml/l - 15.68 ml/l, phosphate from 0.02  $\mu$  mol - 3.23  $\mu$  mol, nitrate from 0.15  $\mu$  mol - 2.58  $\mu$  mol and nitrite from 0.01  $\mu$  mol - 0.85  $\mu$  mol.

13. A method as claimed in claim 1 wherein the micro-propagules in step (d) are clonally propagated through somatic embryogenesis of pigmented filamentous callus.

14. A method as claimed in claim 1 wherein the young plantlets in step (f) are grown in protective cultures in the sea for a period of at least 60 days in submerged transparent polyethylene bags with perforations, attached to floating long lines.

30

Sub  
A<sup>3</sup>

A 3

15. A method as claimed in claim 1 wherein the process of formation of somatic embryos through somatic embryogenesis of pigmented callus is further enhanced by addition of plant growth regulators such as  $\alpha$ -naphthalene acetic acid and 6-benzylaminopurine.

5 16. A method as claimed in claim 1 wherein the harvesting period of at least 60 days can yield a higher biomass or wherein the biomass yield can be maintained constant and the cultivation period reduced.

10 17. A method as claimed in claim 1 wherein two fold increase in growth (fresh weight) is achieved over control parent plants, without change in product yield (carrageenan) and gel strength, through micro-propagules formation from pigmented calli.

add  
A4